Journal of Medical Virology 50:272–275 (1996)

Haemolysis in Hepatitis A Virus Infections Coinciding With the Occurrence of Autoantibodies Against Triosephosphate Isomerase and the Reactivation of Latent Persistent Epstein-Barr Virus Infection

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Haemolysis has been observed frequently as a complication of acute hepatitis A virus (HAV) infection. However, the pathogenic mechanism has not been elucidated completely. In individual cases the detection of anti-erythrocyte antibodies of unknown specificity was described. The raised serum IgM fraction was shown to consist partially of autoantibodies. Previously, we detected autoantibodies of immunoglobulin class M directed against triosephosphate isomerase (IgM anti-TPI) in patients with infectious mononucleosis. These autoantibodies are able to induce haemolysis.

In this study the occurrence of IgM anti-TPI in acute HAV infections and other viral diseases has been investigated. In 33 of 134 patients suffering from HAV infection (IgM anti-TPI was detected. Haematological and chemical data were available from seven of these 33 patients. Mild-to-moderate signs of haemolysis correlating with the IgM anti-TPI titre in the follow-up examinations were demonstrated. The presence of IgM anti-TPI in HAV infections is connected with a reactivation of a latent persistent EBV infection. In other viral infections both the detection of IgM anti-TPI and evidence of a reactivated EBV infection is rare. Thus, we anticipate that IgM anti-TPI antibodies occurring with the reactivation of a latent persistent EBV infection take part in provoking haemolysis in acute HAV infections.

KEY WORDS: hepatitis A virus infection, autoantibodies, latent persistent Epstein-Barr virus

INTRODUCTION

Haematological disturbances in acute hepatitis A virus infection range from mild signs of haemolysis such as anisocytosis, poikilocytosis, and raised reticulocyte counts to rare severe cases of haemolytic anaemia [Hara et al., 1985; Gundersen et al., 1989; Lyons et al., 1990]. Pancytopenia is also known as a complication and is assumed to be due to a direct effect of the virus on the bone marrow [Vallbrecht et al., 1993]. Little is known about the mechanisms of haemolysis. Previously, we detected autoantibodies of the IgM isotype directed against TPI (IgM anti-TPI), an enzyme of the Embden-Meyerhoff pathway, in a patient with acute HAV infection [Ritter et al., 1994]. There was a correlation between the IgM anti-TPI titre and the laboratory data of haemolysis in the follow-up examinations.

The haemolytic effect of IgM anti-TPI on erythrocytes had first been described in patients with infectious mononucleosis [Ritter et al., 1990a,b; Geurs et al., 1992].

The aim of this study was to elucidate the frequency of positive findings for IgM anti-TPI in acute HAV infections and to examine the correlation between the occurrence of the autoantibodies and the signs of haemolysis. Furthermore, the coincidence of the autoantibody with the reactivation of a latent persistent EBV infection was investigated in 134 patients with hepatitis A virus infection and in 127 patients with other viral infections.

PATIENTS AND METHODS

Sera from 134 patients with serologically confirmed diagnosis of acute HAV infection from the serum archive of the diagnostic section of the department were available. Samples were obtained from both outpatient and inpatient departments covering the whole area of South Lower Saxony. As controls, sera from 152 blood donors were examined. One hundred twenty-seven patients with other acute viral infections were examined: hepatitis B (35), hepatitis Delta (2), rubella (44), herpes simplex virus infection (10), varicella (24), and measles (12).

Antibodies to the Epstein-Barr virus capsid antigen (IgG anti-VCA), to the Epstein-Barr nuclear antigen

Accepted for publication July 5, 1996.

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(anti-EBNA), and to the early antigen (anti-EA^d) were determined by immunofluorescence for IgG anti-VCA [Henle and Henle, 1966] and anti-EA^d [Henle et al., 1971] and for anti-EBNA [Reedman and Klein, 1973]. Previous EBV infection was diagnosed by a positive anti-EBNA and/or a positive IgG anti-VCA. IgM anti-HAV and other virus-specific antibodies were measured by commercially available tests (Behring AG, Marburg, FRG). Total IgM was determined by nephelometry; the Coombs test was carried out as described by Lenhard et al. [1978]. IgM anti-TPI was determined as described previously [Ritter et al., 1990a].

RESULTS

Antibodies against TPI belonging to the immunoglobulin class M (IgM anti-TPI) were detectable in sera from 33 out of 134 patients with acute HAV infections. In seven more severely ill patients who were IgM anti-TPI positive, we were able to study the correlation between the serological data and the chemical and haemotological values in follow-up investigations as shown in Table I.

Autoantibodies against TPI reach their peak parallel with the maximum rise of the ALAT. The lowest haemoglobin levels were observed in the 2nd week of clinical HAV infection. Haptoglobin was decreased, and reticulocytosis of 4–5% was found in the four patients examined. There were no data on the osmotic resistance and LDH isoenzymes available. The total IgM fraction was increased more than twofold. In patients 5 and 6 the anti-IgG Coombs test was negative and the anti-C3 Coombs test was positive. Coombs tests were undertaken only in these two patients. Anti-I/i cold agglutinins were not detectable. In all patients observed haemoglobin levels were gradually returning to normal after 3 weeks, while IgM anti-TPI levels were falling. IgM anti-TPI was no longer detectable after 2 months. An antibody switch from IgM to IgG anti-TPI did not take place.

Sera were investigated for EBV-specific antibodies, since IgM anti-TPI had first been discovered in patients with acute EBV infections [Ritter et al., 1990b]. Furthermore, we examined sera from patients with other acute viral diseases for the presence of IgM anti-TPI and EBV-specific antibodies (Table II).

Previous EBV infection was diagnosed in all of the 33 patients whose sera were positive for IgM anti-TPI. The presence of IgM anti-TPI was connected with a positive anti-EA^d as a marker of a reactivated EBV infection in 27 cases. The anti-EA^d assay was only positive in the presence of IgM anti-TPI.

In the other viral infections the detection of IgM anti-TPI and anti-EA^d was a rare finding. In sera of two patients with rubella, one patient with varicella, and two blood donors IgM anti-TPI and anti-EA^d were found in cut-off values. Haematological data and follow-up sera of these patients were not available. In the control group "blood donors" two sera out of 152 were positive for IgM anti-TPI. An acute EBV infection was diagnosed in the one positive blood donor who was also anti-EA positive; the other positive donor could not be followed-up.

DISCUSSION

A haemolytic state of varying degree is a common complication of acute HAV infections, but the pathogenic mechanism has remained unexplained. A shortened erythrocyte survival time points to an immune reaction as the causative mechanism [Conrad, 1969]. A number of antibodies were detected in the course of acute HAV infections, but none of these antibodies could be related to the haemolytic phenomena [Horejsi et al., 1970]. On the other hand, the specificity of antibodies found to be responsible for haemolysis was not known [Conley et al., 1982; Hara et al., 1985; Gundersen et al., 1989, Lyons et al., 1990].

The detection of IgM anti-TPI during acute hepatitis A infection is striking as the specificity of the autoantibody had been elucidated in contrast to other antibodies inducing haemolysis in acute HAV infections. IgM anti-TPI was first detected in patients with infectious mononucleosis [Ritter et al., 1990b], and was shown to be a cause of haemolysis [Ritter et al., 1990b,c; Geurs et al., 1992]. Affinity-purified IgM anti-TPI was observed to bind to erythrocytes and to induce an increased chromium-51 release from the marked erythrocytes whilst activating complement [Ritter et al., 1990c; Geurs et al., 1992]. The autoantibody was shown to inhibit the enzyme function of TPI in a patient with acute HAV infection [Ritter et al., 1994]. This is of importance since TPI deficiencies are known to cause haemolytic anaemia [Maquat et al., 1985]. Being part of the glycolytic enzyme complex TPI is bound to the cytoplasmatic domain of the anion-transport protein in direct contact with Na^+/K^+-ATPase [Holan et al., 1995]. During the course of physiological ageing parts of the TPI may reach the outer membrane, where they are recognized by the autoantibodies. Initial defects of the membrane may allow the antibody to enter the cell and bind to TPI, leading to an inhibition of the enzyme function. In the patient described previously, who developed haemolysis in the course of acute hepatitis A virus infection, both the haemolytic effect of the affinity-purified antibody and its ability to inhibit the enzyme function were demonstrated [Ritter et al., 1994].

The high rate of appearance of IgM anti-TPI demonstrated and the connection with reactivated EBV infections is unique to HAV infections as shown by examination of samples from patients with other viral infections including hepatitis B virus infection. The data demonstrate that the simultaneous occurrence of IgM anti-TPI and haemolysis is not uncommon in patients with acute hepatitis A virus infection. A reactivated EBV infection seems to trigger the production of IgM anti-TPI. Its detection coincided with a positive anti-EA^d in 27 out of 33 cases. The rise of anti-EA^d gives an important serological indicator of the activity of EBV, although it is not observed in all cases of reactivated EBV infection. IgM anti-TPI often preceeds the appearance of anti-EA^d in cases of reactivation as we noticed in large-scale routine examinations. Thus, we assume that there is also an activity of EBV in the six patients negative for anti-

TABLE I. Laboratory Data of Seven Patients With Acute HAV Infection and Haemolysis*

Patient (sex, age in years)	ALAT (U/l)	Hb (g/dl)	Haptoglobin (g/l)	IgM anti-TPI (titre)	anti-EA[d] (titre)
Patient 1 (M, 36)					
Day of diagnosis	141	13,9	n.d.	1,600	160
Day 3	1016	12,9	n.d.	2,400	160
Day 10	447	10,8	0.1	1,200	n.d.
Day 24	62	11,8	0.8	400	40
Patient 2 (F, 34)					
Day of diagnosis	294	12.8	n.d.	2,400	320
Day 3	985	12.4	n.d.	1,600	640
Day 10	312	11.1	0.5	800	n.d.
Day 31	43	12.3	0.7	200	160
Day 56	28	13.9	n.d.	<50	<40
Patient 3 (M, 10)					
Day of diagnosis	520	13.2	n.d.	1,600	160
Day 2	534	12.7	0.6	1,600	n.d.
Day 9	128	11.7	0.4	800	n.d.
Day 23	22	11.9	0.6	200	80
Day 47	15	12.5	n.d.	<50	<40
Patient 4 (F, 23)					
Day of diagnosis	628	12.4	n.d.	3,200	640
Day 4	582	11.8	n.d.	3,200	n.d.
Day 11	126	10.5	0.0	1,600	320
Day 25	46	11.2	0.3	800	160
Day 56	32	12.3	1.3	<50	<40
Patient 5 (M, 26)					
Day of diagnosis	416	12.8	n.d.	1,600	640
Day 3	912	10.1	n.d.	3,200	n.d.
Day 9	298	9.9	0.0	1,600	n.d.
Day 17	44	12.1	0.3	400	160
Day 35	22	13.2	1.0	100	80
Day 41	25	13.9	1.1	<50	40
Patient 6 (M, 20)					
Day of diagnosis	594	13,3	n.d.	3,200	320
Day 2	578	11.4	0.0	6,400	n.d.
Day 9	157	9.8	0.0	1,600	n.d.
Day 18	78	10.9	0.3	1,200	160
Day 28	52	12.1	0.7	400	160
Day 110	18	14.6	n.d.	<50	<40
Patient 7 (F, 30)					
Day of diagnosis	608	13.1	n.d.	1,200	640
Day 3	459	12.5	n.d.	1,600	n.d.
Day 9	82	10.2	0.0	1,200	320
Day 16	28	9.6	0.0	800	n.d.
Day 31	18	10.8	0.3	200	80

*n.d. = not done. Normal values: ALAT 5-21 U/l (1 U/l = 16.6 nkat/l), haemoglobin (Hb) 12-16 g/dl, haptoglobin 0.7-3.2 g/l, IgM anti-TPI antibody titre <50, anti-EA[d] ≤40.

TABLE II. Detection of IgM Anti-TPI and EBV-Specific Antibodies in Patients With Different Viral Infections and Controls

Infectious agent	Samples tested (N)	IgG anti-VCA/ anti-EBNA positive	IgM anti-TPI positive	anti-EA[d] positive
HAV	134	116	33	27
HBV	35	35	0	0
HDV	2	2	0	0
Rubella virus	44	30	2	2
HSV	10	9	0	0
VZV	24	16	1	1
Measles virus	12	5	0	0
Control group:				
Blood donors	152	10	2	1

EA^d, which could not be confirmed by further testing since sera for follow-up examinations were not available.

The pathogenic principle of IgM anti-TPI should be taken into account in acute HAV infections complicated by haemolysis, and testing for reactivated EBV infections is recommended. The production of the autoantibody is self-limited. However, plasmapheresis proved helpful in a patient with a prolonged course of haemolysis in infectious mononucleosis, whereas corticosteroid therapy had no effect [Geurs et al., 1992]; this should be recognized in the rare severe cases of haemolysis in HAV infections.

Furthermore, we anticipate that the reactivation of a latent persistent EBV infection during the course HAV infection may also play a role in cases of protracted hepatocellular disease.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (Ri 677/1-2).

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